Placenta 33 (2012) 682–687

Contents lists available at SciVerse ScienceDirect

Placenta

journal homepage: www.elsevier.com/locate/placenta

Cytokines and chemokines production by mononuclear cells from parturient women after stimulation with live *Toxoplasma gondii*

K. Rezende-Oliveira [a,*], N.M. Silva [b,1], J.R. Mineo [c,2], V. Rodrigues Junior [d,3]

[a] Universidade Federal de Uberlândia, Faculdade de Ciências Integradas do Pontal, Rua 20, 1600, Bairro Tupã, Ituiutaba 38304-402, Minas Gerais, Brazil
[b] Universidade Federal de Uberlândia, Instituto de Ciências Biomédicas, Av. Pará, 1720, Bloco 2B, Bairro Umuarama, Uberlândia 38400902, Minas Gerais, Brazil
[c] Universidade Federal de Uberlândia, Instituto de Ciências Biomédicas, Av. Pará, 1720, Bloco 4C, Bairro Umuarama, Uberlândia 38400902, Minas Gerais, Brazil
[d] Universidade Federal do Triângulo Mineiro, Laboratório de Imunologia, Av. Frei Paulino, 30, Bairro Abadia, CEP 38025-180, Uberaba, Minas Gerais, Brazil

ARTICLE INFO

Article history:
Accepted 30 May 2012

Keywords:
Pregnancy
Immune response
Toxoplasma gondii

ABSTRACT

Toxoplasma gondii is an obligate intracellular parasite that can cause variable clinical symptoms or can even be asymptomatic in immunocompetent individuals. More severe symptoms are observed in immunocompromised patients and congenital transmission of the parasite has been reported. The objective of this study was to evaluate the response of peripheral blood mononuclear cells (PBMC) in parturient and non-pregnant women exposed to live tachyzoites of *T. gondii* strain RH or ME49. PBMC were isolated from parturient and non-pregnant women with negative or positive serology for toxoplasmosis and cultured with live tachyzoites of the two *T. gondii* strains for 24 h. Next, the cell culture supernatants were collected and levels of CCL2, CCL5, IL-6, IL-10, IL-12, and TNF-α produced by PBMC after tachyzoite exposure were measured. Live tachyzoite forms of *T. gondii* significantly inhibited the synthesis of CCL2 in seropositive parturient women, whereas a stimulatory effect on CCL5 was observed in seronegative parturient women. Cells from *T. gondii*-seronegative non-pregnant women produced significantly higher levels of TNF-α and IL-12, demonstrating the proinflammatory profile induced by the presence of the parasite in culture. The results suggest that the immunomodulation seen during pregnancy contributes to the development of an environment that facilitates escape of the parasite from the immune response.

1. Introduction

Toxoplasma gondii is an obligate intracellular parasite and the causative agent of toxoplasmosis, a cosmopolitan and opportunistic infection that manifests particularly in immunosuppressed patients [1], but is asymptomatic in immunocompetent individuals. However, in women who become infected during pregnancy the disease can be transmitted through the placenta, causing abortion or fetal disorders (retinochoroiditis, cerebral calcification, seizures, and hydrocephalus). Toxoplasmosis is therefore of clinical importance for parturient women and children with congenital infection [2,3].

The capacity of *T. gondii* to cross biological barriers such as the intestinal epithelium, blood–brain barrier or placenta is an important factor for the success of infection and is directly related to the genotype of the parasite [4]. A study investigating *T. gondii* strains isolated from patients with acquired immunodeficiency syndrome (AIDS) and patients with congenital toxoplasmosis demonstrated the predominance of genotype II [5,6].

In the initial infection with *T. gondii* some different mechanisms of innate immune response are trigger. Toll-like receptors (TLRs) are members of a family of proteins that recognize different types of antigens. Different TLRs present on leukocytes and trophoblast cells transduce intracellular signals that lead to the transcription of chemokine and proinflammatory cytokine genes [7,8]. Studies have demonstrated that IL-12, IL-10 and TNF-α play an important role in the innate immunity against *T. gondii* and influence the adaptive immune response. These cytokines are important immunomodulators that act during the early stage of infection [9,10].

The main mechanism involved in the clearance of intracellular parasites is the development of a Th1-type immune response by the host. In this response, the main cells responsible for parasite

* Corresponding author. Tel.: +55 34 3271 5240; fax: +55 34 3318 5651.
E-mail addresses: karinerezende.oliveira@gmail.com (K. Rezende-Oliveira), nmsilva@icbim.ufu.br (N.M. Silva), jrmineo@ufu.br (J.R. Mineo), vrodrigues@mednet.com.br (V. Rodrigues Junior).
[1] Tel.: +55 34 3218 2240; fax: +55 34 3218 2333.
[2] Tel.: +55 34 3218 2195; fax: +55 34 3218 2333.
[3] Tel.: +55 34 3318 5289; fax: +55 34 3312 1487.

0143-4004
http://dx.doi.org/10.1016/j.placenta.2012.05.013

elimination are polymorphonuclear neutrophils, dendritic cells and macrophages that produce cytokines such as TNF-α and IL-12 which, in turn, stimulate natural killer cells to produce IFN-γ [11]. IL-12 is mainly synthesized by macrophages and dendritic cells during stimulation with *T. gondii* antigens and plays an important role during the acute phase of infection. TNF-α is produced by monocytes/macrophages, T lymphocytes and mast cells and stimulates the microbicidal activity of these cells, acting synergistically against *T. gondii* [12,13]. Furthermore, monocytes/macrophages, dendritic cells and fibroblasts can produce IL-6, a cytokine that acts synergistically with TNF-α. This cytokine induces the differentiation of B lymphocytes into plasma cells and the differentiation of cytotoxic T cells [14].

Chemokines are essential for the recruitment of neutrophils, macrophages and T cells to the site of infection with *T. gondii* and their receptors play an important role in innate immunity [15]. The CCR5 receptor interacts with CC chemokines, such as CCL5, CCL3 and CCL4 [16]. *In vitro*, *T. gondii* induces the synthesis of different chemokines of the CC (CCL2 and CCL5) and CXC (CXCL9, CXC-10s and CXCL8/IL-8) groups by different cell types [9,16,17]. *T. gondii* tachyzoites determine the intensity of chemokine synthesis. In this respect, parasites of strain RH have been shown to induce the expression and secretion of CCL2 after infection of human fibroblasts, suggesting that tachyzoites participate in the induction of chemokines and in the recruitment of cells during infection [18].

During pregnancy, the immune system of the mother presents a Th2-type response characterized by the production of cytokines such as IL-4, IL-5, IL-10, and TGF-β [19,20]. This Th2 deviation contributes to the maintenance of the corpus luteum, adhesion of the blastocyst to the endometrial wall and fetal development, and participates in cell differentiation and fetal tolerance during pregnancy [20]. During the first and second trimester of gestation, the human trophoblast synthesizes high levels of IL-10 and expresses the IL-10 receptor. However, these levels decline during the third trimester [21].

The correlation between peripheral blood and uterine leukocyte activities is dependent of the type of disease that it is investigating. In this context, the mucosal immune responses towards *Chlamydia trachomatis* are different from those of PBMCs, being this data important to understand the cytokine responses in the female genital tract during chlamydial infection [22]. In contrast, there is relevant information concerning the influence of systemic response in uterine immune response in other pathologies, such as HPV infection, being lower IFN-γ production by PBMC correlated with cervical cancer severe disease induced by this virus [23]. Related to human toxoplasmosis, however, there is a lack of information establishing a clear functional relationship between systemic and uterine leukocytes. Thus, the aim of the present study was to evaluate the chemokines and cytokines production of peripheral blood mononuclear cells (PBMC) from parturient and non-pregnant women exposed to live tachyzoites of RH or ME49 *T. gondii* strains.

2. Materials and methods

2.1. Parasites

Tachyzoites of *T. gondii* strains RH and ME49 were used in the present investigation and were maintained in human foreskin fibroblasts (HFF) cultured in RPMI 1640 medium (Gibco BRL, Grand Island, NY, USA) supplemented with 5% fetal bovine serum (Gibco BRL) inactivated by heating and 80 mg/L garamycin (Schering-Plough, Brazil). The parasites were obtained in culture by 3–4 passages in confluent monolayers of HFF, with an infection rate of three tachyzoites per cell. After lysis of infected cells, the culture supernatant was collected and centrifuged at 50× *g* for 2 min. Next, the cellular debris-free tachyzoites enriched supernatant was centrifuged at 500× *g* for 10 min and the pellet was resuspended in RPMI 1640 medium supplemented with 25 mM HEPES (Sigma Chemical Co., St Louis, MO, USA), 80 mg/L garamycin (Schering-Plough, Brazil) and 5% heat-inactivate fetal bovine serum (Gibco BRL, Grand Island, NY, USA) to obtain tachyzoite-enriched preparations.

2.2. Isolation of peripheral blood mononuclear cells from parturient and non-pregnant women with negative or positive serology for toxoplasmosis

About 10 ml of blood was collected from seven parturient (during labor) and seven non-pregnant women who were negative for *Toxoplasma* IgG antibody and from 10 parturient and six non-pregnant women who were IgG positive. The parturients were recruited from the Teaching Hospital of Universidade Federal do Triângulo Mineiro and Hospital Beneficencia Portuguesa, Uberaba city, Minas Gerais state, Brazil. The non-pregnant women were volunteers from Universidade Federal do Triângulo Mineiro. The subjects ranged in age from 20 to 45 years. For the confirmation of serology, plasma was collected from the patients for the detection of IgM and IgG antibodies by chemoluminescence (Siemens Immunolite®, Ireland). All subjects were negative for anti-*T. gondii* IgM antibodies, characterizing the chronic phase of infection. All patients selected in this study had no diagnosis for other chronic or acute infection/inflammatory disease according to the clinical profile records, nor received any specific drug treatment.

Peripheral blood was centrifuged on a Ficoll-Hypaque® (1.078 g/mL) (GE Healthcare, Piscataway, NJ, USA) gradient and fraction of mononuclear cells adjusted to 2×10^6 cells/well were plated in 24-well plates and cultured at 37 °C in a 5% CO_2 atmosphere. The study was approved by the Ethics Committee of Universidade Federal do Triângulo Mineiro (protocol No. 0906).

2.3. Detection of cytokines and chemokines in culture supernatants

The 24 h cultures of PBMC were infected with live tachyzoites (2 parasites/cell) of RH or ME49 *T. gondii* strains. After additional 24 h culture, the supernatants were collected for analysis of cytokines and chemokines contents.

Cytokines and chemokines concentrations were measured in the supernatant of PBMC cultures by ELISA (Enzyme-Linked Immunoabsorbent Assay). TNF-α and IL-10 were assayed using the BD OptEIA™ kit (BD Biosciences Pharmingen, San Diego, California), with detection limit of the method of 3.7 and 18 pg/ml, respectively. IL-6, IL-12p70, CCL2 and CCL5 were measured using the RD Systems kit (Minneapolis, USA), with detection limit of the method of 12.6, 6.7, 1.5 and 0.9 pg/ml, respectively.

2.4. Statistical analysis

The nonparametric Mann–Whitney test was used for comparison between two groups (parturient and non-pregnant women or *T. gondii*-seropositive and -seronegative women). Two variables were compared within the same group by the Wilcoxon test (unstimulated and *T. gondii*-stimulated). The Statview (ABACUS) for Windows program was used for statistical analysis. A p value <0.05 was considered to be statistically significant.

3. Results

3.1. PBMC chemokines production stimulated by T. gondii

Low CCL2 levels were observed in the supernatants of PBMC cells from both seronegative and seropositive parturients infected with ME49 strain (Fig. 1a). In non-pregnant women, low levels of this chemokine were seen in the seronegative group under ME49 infection (Fig. 1a). In addition, higher levels of CCL2 were observed in *T. gondii*-seropositive non-pregnant women after the addition of ME49 strain when compared with the group of seropositive parturients (Fig. 1a). RH strain negatively regulated the CCL2 production in cell cultures of seronegative parturient women; and also in the PBMC from non-pregnant women, irrespective of *T. gondii* serology (Fig. 1a).

The production levels of CCL5 by PBMC were higher than that of CCL2. Although the production of CCL5 was higher in seronegative parturient women, no significant difference was observed when compared to the seropositive parturient group. In parturient women group, the addition of *T. gondii* tachyzoites to the culture did not significantly modify the production of CCL5 (Fig. 1b). The CCL5 levels were significantly higher in seropositive non-pregnant women compared to seropositive parturient women after stimulation with the two strains (Fig. 1b). In contrast to CCL2 levels, the addition of RH or ME49 strain tachyzoites induced a significant increase in the synthesis of CCL5 by cells from seropositive non-pregnant women compared to uninfected cells (Fig. 1b).

No significant difference in CCL2 or CCL5 levels was observed between parturient and non-pregnant women when only seronegative individuals were compared (Fig. 1a and b).

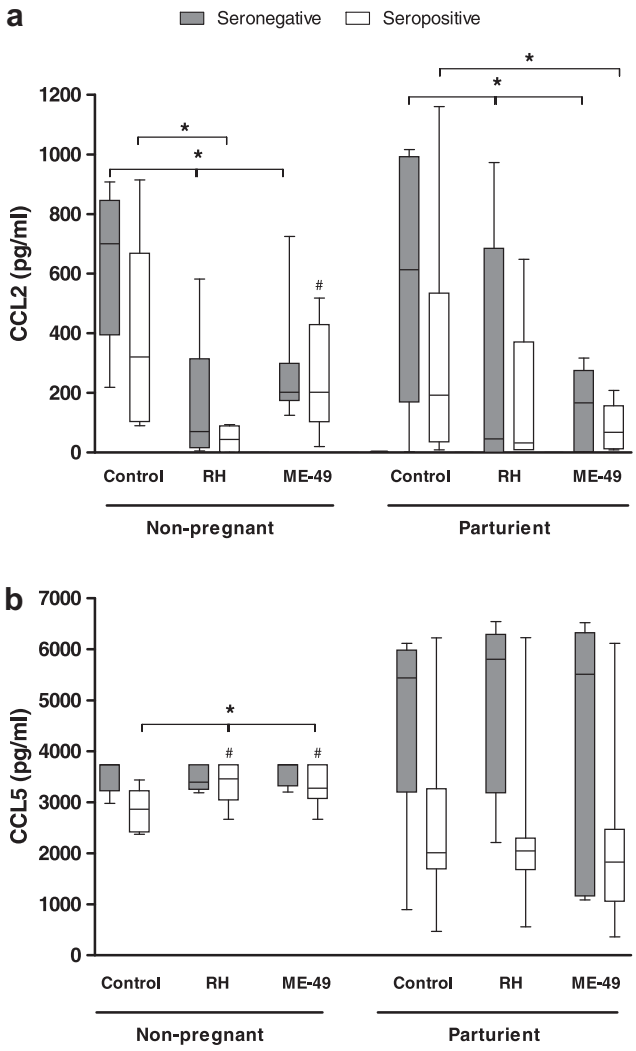

Fig. 1. Comparison of chemokine production by PBMC in 24 h culture supernatants stimulated with RH or ME49 strains of *Toxoplasma gondii*. Non-pregnant or parturient women were grouped as *T. gondii*-seronegative (gray bar) or -seropositive (white bar). The levels of CCL2 (a) or CCL5 (b) were measured by enzyme immunoassay (ELISA). The horizontal line indicates the median, bars the 25% and 75% percentiles, and vertical lines the 10% and 90% percentiles. (*$p < 0.05$, Wilcoxon test); #significant differences between seropositive non-pregnant and parturient women's PBMC stimulated with the same *T. gondii* strain ($p < 0.05$, Mann–Whitney test).

3.2. PBMC cytokines production stimulated by T. gondii

TNF-α levels were significantly higher in the non-pregnant group than in the parturient group after stimulation with tachyzoites of *T. gondii* strains RH or ME49, irrespective of parasite serology (Fig. 2a). In parturient's PBMC, the addition of tachyzoites of ME49 strain resulted in a significant increase of TNF-α levels in the seronegative group, whereas no significant difference was observed in the seropositive group (Fig. 2a). In non-pregnant women, the addition of ME49 strain tachyzoites induced a significant increase of TNF-α irrespective of *T. gondii* serology. RH strain positively modulated the synthesis of TNF-α only in cells from seronegative non-pregnant women (Fig. 2a).

No significant difference in the levels of IL-10, IL-12 or IL-6 was observed between seronegative and seropositive parturient women, even after the addition of live tachyzoites (Fig. 2b–d). In contrast, in non-pregnant women the addition of tachyzoites of ME49 strain induced a significant increase of IL-10 and IL-6 in the seronegative group and of IL-12 in the seronegative and seropositive groups (Fig. 2b–d). Only the group of seronegative non-pregnant women responded with a significant increase of IL-10 after the addition of tachyzoites of RH strain (Fig. 2b).

The IL-10, IL-12 and IL-6 levels were also compared between parturient and non-pregnant women according to the serology. Higher IL-12 levels were observed in the cell culture supernatants of seropositive non-pregnant women stimulated with ME49 strain when compared to seropositive parturient women (Fig. 2c). Additionally, higher IL-12 levels were observed in supernatant of non-stimulated, RH or ME49-stimulated cell cultures from non-pregnant seronegative compared to seronegative parturient group (Fig. 2c). Higher IL-6 levels were observed in unstimulated cultures from seropositive non-pregnant women compared to seropositive parturient women (Fig. 2d).

4. Discussion

The early pregnancy is characterized by a proinflammatory profile with high levels of CXCL8/IL-8, CCL2, CCL5, and Granulocyte Colony-Stimulating Factor (G-CSF) [24]. The levels of these cytokines decline during the second trimester, but increase again at the end of the gestational period. Parturition is characterized by the migration of immune cells to the myometrium that promote the onset of an inflammatory process related to labor [25]. In addition, it was previously shown that chemokines are important for leukocyte recruitment to the human endometrium at the times of pregnancy and menstruation [24,25]. The protozoan *T. gondii* infects a wide variety of hosts but generally has few clinical consequences. However, infection of mothers during pregnancy, especially in the third trimester, poses a major risk of vertical transmission and may cause important clinical sequelae in the newborn infant [1,26].

T. gondii tachyzoites were found to modulate the synthesis of CCL2 in cell cultures of parturient and non-pregnant women. The presence of tachyzoites in the cultures negatively modulated the synthesis of CCL2, irrespective of parasite strain and *Toxoplasma* seronegative parturient, when compared to unstimulated cultures. When PBMC from seropositive parturient women were analyzed, only ME49 tachyzoites-stimulated cells presented a decreasing in CCL2 levels compared with unstimulated cells. Additionally, decreased CCL2 levels were found in seropositive parturient women compared to non-pregnant women infected with strain ME49.

CCL2 is a chemokine produced by macrophages, dendritic cells, and endothelial cells after stimulation with cytokines such as TNF-α and IL-1. The CCL2 receptor is generally expressed on monocytes and effector and memory T cells, including Th1 and Th2 cells [27,28]. CCL2 has been shown to be involved in the recruitment of these cells to the sites of infection with *T. gondii* [17]. The tachyzoite soluble antigen is able to activate the cascade of CCL2 synthesis in the host cell through TLR2 [29]. Infection of human fibroblasts with RH strain was found to significantly increase the expression and synthesis of CCL2 by these cells [18]. In the present work, the negative modulation of CCL2 production by PBMC may contribute to the escape mechanisms used by the parasite.

In the present study, it was observed higher CCL5 production by PBMC from seropositive non-pregnant compared with parturient women under *T. gondii* infection. The CCL5 receptor, CCR5, plays a potential role in the maintenance of homeostasis during pregnancy. In the parturient uterus and placenta, various forms of regulatory T cells (CD4+ CD25+ Foxp3+) express CCR5 in response to paternal alloantigens [30]. The number of these cells is increased in peripheral blood of allogeneically pregnant mice and humans. The presence of these cells in the uterus and placenta may favor

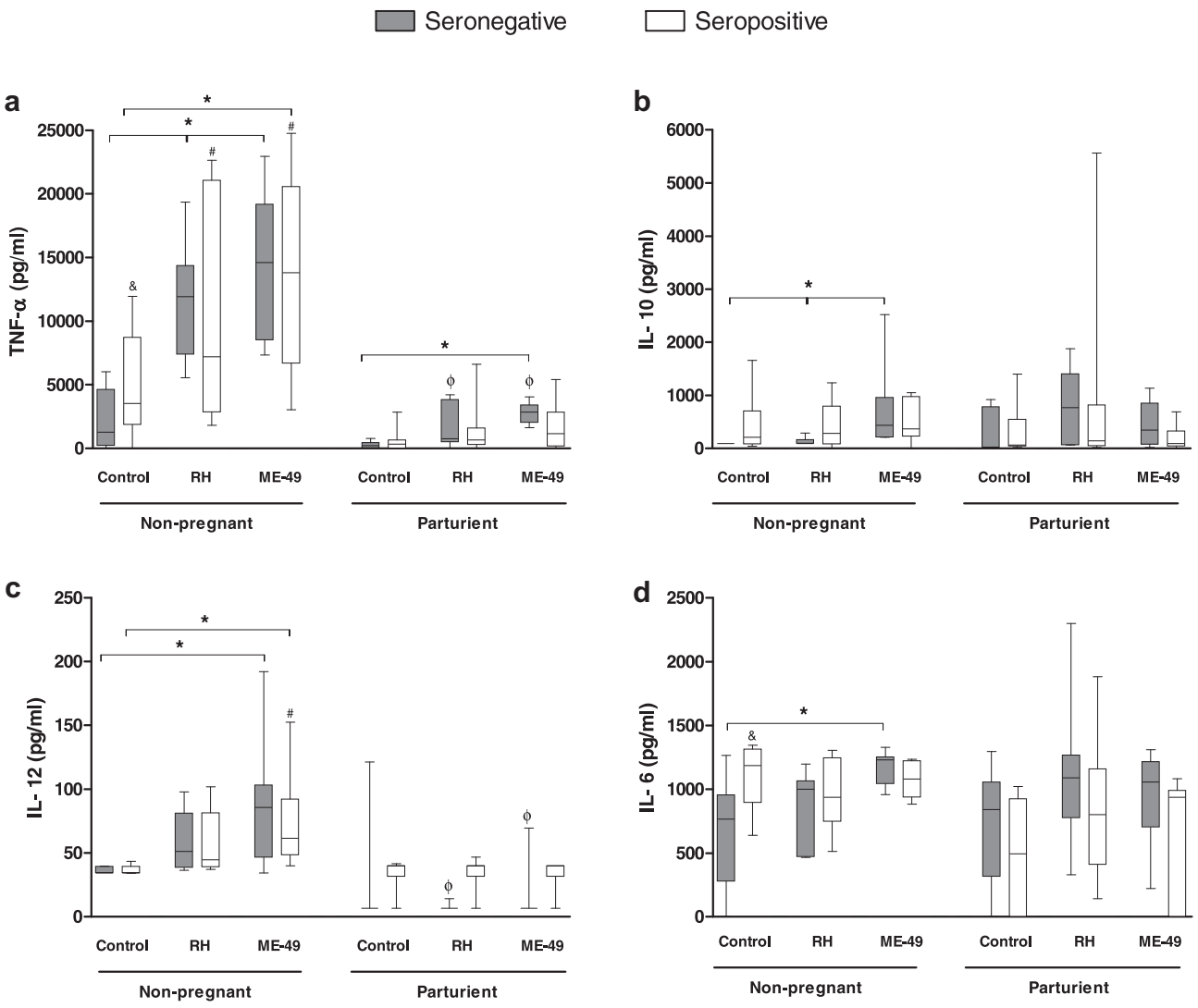

Fig. 2. Comparison of cytokine production by PBMC in 24 h culture supernatants stimulated with RH or ME49 strains of *Toxoplasma gondii*. Non-pregnant or parturient women were grouped as *T. gondii*-seronegative (gray bar) or -seropositive (white bar). The levels of TNF-α (a), IL-10 (b), IL-12 (c) or IL-6 (d) were measured by enzyme immunoassay (ELISA). The horizontal line indicates the median, bars the 25% and 75% percentiles, and vertical lines the 10% and 90% percentiles. (*$p < 0.05$, Wilcoxon test); #significant differences between seropositive non-pregnant and parturient women's PBMC stimulated with the same *T. gondii* strain; &significant differences between seropositive non-pregnant and parturient women's PBMC cultured with medium alone; ϕSignificant differences between seronegative non-pregnant and parturient women's culture supernatants stimulated with the same *T. gondii* strain ($p < 0.05$, Mann–Whitney test).

gestation. Progesterone downregulates the expression of CCR5 during pregnancy, but the expression of this receptor increases close to the time of labor [31]. IL-12-inducing activity is seen in secreted *T. gondii* protein suspensions and cyclophilin-18 is one of the components that bind directly to human and mouse CCR5, with affinities comparable to that of the prototypical ligand, CC chemokine ligand 4 (CCL4) [32]. Studies have shown that *T. gondii* promotes the migration of inflammatory cells through the induction of chemokines and their receptors, with the parasite producing substances that stimulate the synthesis of cytokines such as IL-12 and of CCL5 through the CCR5 receptor [32]. Oral infection with *T. gondii* ME49 strain has been shown to increase CCL5 and CCR5 mRNA expression in intestinal epithelial cells of C57BL/6 mice on day 8 of infection [33] and CCL5 mRNA expression in the brain 10 days after parasite inoculation [34]. The present results showed significantly lower production of CCL5 by PBMC in *T. gondii*-seropositive parturient women after stimulation with strain RH or ME49 when compared to the seropositive non-pregnant group. These findings suggest that the immune response modulation induced by gestation may contribute to a decrease in CCL5 levels, favoring infection with *T. gondii*. Since all seropositive patients were in the chronic phase of infection, the lower production of this chemokine in the seropositive parturient group might have impaired the immune mechanisms involved in parasite control.

The present study analyzed cytokine levels in the cell culture supernatants of parturient and non-pregnant women after 24 h of incubation with two different *T. gondii* strains. TNF-α and IL-12 levels were significantly elevated in the cell culture supernatants of non-pregnant women infected or not with either *T. gondii* strain. In contrast, TNF-α and IL-12 production was decreased in parturient women. Monocytes of pregnant women may enhance IL-12 production *in vitro* when compared to monocytes of non-pregnant women [35]. The present results obtained for parturient women suggest that the parasite modulates IL-12 production, controlling the immune response for its survival.

Expression of indoleamine 2,3-dioxygenase, an enzyme involved in the first steps of tryptophan metabolism which is important for tachyzoites replication, is modulated by TNF-α,

contributing to the control of parasite burden [36]. The present results suggest that the decreased levels of this cytokine in parturient women may contribute to tachyzoite invasion and toxoplasmosis development.

Previous experiments have shown that neutralization of endogenous TNF-α during chronic toxoplasmosis results in lethal exacerbation of the disease [37]. In addition, TNF receptor p55 and p75 (TNFRp55-/- and p75-/-) and TNFRp55 (TNFRp55-/-)-deficient mice develop lethal toxoplasmic encephalitis [38,39], a finding demonstrating the crucial role of TNF-α in the control of the parasite during chronic infection. Additionally, IL-12 is essential for cell-mediated immunity to *T. gondii* infection [40]. In this respect, the present investigation suggests that the modulation of the immune response induced by gestation may favor parasite proliferation. In contrast, IL-6 and IL-10 levels were similar in the two groups of women studied. The addition of tachyzoites of RH or ME49 strain to PBMC cultures of seronegative non-pregnant women resulted in a higher production of IL-10 compared to unstimulated cells. Despite the lack of a significant difference between groups, cells from parturient women produced high levels of IL-10 under these culture conditions, irrespective of positive or negative serology. IL-10 is a cytokine that modulates the innate and adaptive immune response [41]. In an *in vitro* study, addition of exogenous IL-10 to BeWo trophoblast cell cultures induced a small increase in *T. gondii* intracellular replication [42]. Thus, IL-10 may facilitate passage of the parasite through the placenta.

Parturient women have been shown to present a Th2/regulatory T cell response pattern [19,20] and IL-10 levels decline in the last trimester of gestation [21]. The induction of IL-10 synthesis by the parasite is considered to be an escape mechanism from the host immune response. However, some investigators believe that this synthesis is not directly related to parasite virulence and that the high production of this cytokine is not involved in persistence of the parasite in the host cell [43]. The present study showed that the ME49 strain was able to stimulate the production of IL-10 in mononuclear cell cultures of seronegative non-pregnant women. ME49 is a cystogenic and weakly virulent *T. gondii* strain [4] and stimulation of the production of IL-10, an anti-inflammatory cytokine, may represent an evasion mechanism that contributes to parasite maintenance. Furthermore, type II strain of *T. gondii*, like ME49 parasites, is often vertically transmitted in human pregnancy [44,45].

In conclusion, the present study demonstrated a different immunological profile characterized by a predominantly proinflammatory response in non-pregnant women, irrespective of positive or negative serology for toxoplasmosis. Taken together, these results suggest that the immunomodulation observed during pregnancy creates mechanisms that favor the escape of the parasite and that the parasite itself contributes to this condition by differentially modulating the synthesis of chemokines by PMBC.

Acknowledgment

This work was supported by Brazilian Research Agencies (FAPEMIG, CAPES and CNPq).

References

[1] Tenter AM, Heckeroth AR, Weiss LM. *Toxoplasma gondii*: from animals to humans. Int J Parasitol 2000;30(12–13):1217–58.
[2] Luft BJ, Remington JS. Toxoplasmic encephalitis in AIDS. Clin Infect Dis 1992; 15(2):211–22.
[3] Dubey JP. Toxoplasmosis-a waterborne zoonosis. Vet Parasitol 2004; 126(1–2):57–72.
[4] Saeij JP, Boyle JP, Boothroyd JC. Differences among the three major strains of *Toxoplasma gondii* and their specific interactions with the infected host. Trends Parasitol 2005;21(10):476–81.
[5] Howe DK, Sibley LD. *Toxoplasma gondii* comprises three clonal lineages: correlation of parasite genotype with human disease. J Infect Dis 1995;172(6): 1561–6.
[6] Ajzenberg D, Banuls AL, Tibayrenc M, Darde ML. Microsatellite analysis of *Toxoplasma gondii* shows considerable polymorphism structured into two main clonal groups. Int J Parasitol 2002;32(1):27–38.
[7] Holmlund U, Cebers G, Dahlfors AR, Sandstedt B, Bremme K, Ekstrom ES, et al. Expression and regulation of the pattern recognition receptors toll-like receptor-2 and toll-like receptor-4 in the human placenta. Immunology 2002;107(1):145–51.
[8] Agrawal V, Hirsch E. Intrauterine infection and preterm labor. Semin Fetal Neonatal Med 2012;17(1):12–9.
[9] Bliss SK, Marshall AJ, Zhang Y, Denkers EY. Human polymorphonuclear leukocytes produce IL-12, TNF-alpha, and the chemokines macrophage-inflammatory protein-1 alpha and-1 beta in response to *Toxoplasma gondii* antigens. J Immunol 1999;162(12):7369–75.
[10] Bliss SK, Gavrilescu LC, Alcaraz A, Denkers EY. Neutrophil depletion during *Toxoplasma gondii* infection leads to impaired immunity and lethal systemic pathology. Infect Immun 2001;69(8):4898–905.
[11] Gazzinelli RT, Hakim FT, Hieny S, Shearer GM, Sher A. Synergistic role of CD4+ and CD8+ T lymphocytes in IFN-gamma production and protective immunity induced by an attenuated *Toxoplasma gondii* vaccine. J Immunol 1991;146(1):286–92.
[12] Hunter CA, Bermudez L, Beernink H, Waegell W, Remington JS. Transforming growth factor-beta inhibits interleukin-12-induced production of interferon-gamma by natural killer cells: a role for transforming growth factor-beta in the regulation of T cell-independent resistance to *Toxoplasma gondii*. Eur J Immunol 1995;25(4):994–1000.
[13] Yamamoto JH, Vallochi AL, Silveira C, Filho JK, Nussenblatt RB, Cunha-Neto E, et al. Discrimination between patients with acquired toxoplasmosis and congenital toxoplasmosis on the basis of the immune response to parasite antigens. J Infect Dis 2000;181(6):2018–22.
[14] Filisetti D, Candolfi E. Immune response to *Toxoplasma gondii*. Ann Ist Super Sanita 2004;40(1):71–80.
[15] Del Rio L, Bennouna S, Salinas J, Denkers EY. CXCR2 deficiency confers impaired neutrophil recruitment and increased susceptibility during *Toxoplasma gondii* infection. J Immunol 2001;167(11):6503–9.
[16] Brenier-Pinchart MP, Pelloux H, Simon J, Ricard J, Bosson JL, Ambroise-Thomas P. *Toxoplasma gondii* induces the secretion of monocyte chemotactic protein-1 in human fibroblasts, in vitro. Mol Cell Biochem 2000;209(1–2): 79–87.
[17] Denney CF, Eckmann L, Reed SL. Chemokine secretion of human cells in response to *Toxoplasma gondii* infection. Infect Immun 1999;67(4):1547–52.
[18] Brenier-Pinchart MP, Vigan I, Jouvin-Marche E, Marche PN, Pelet E, Gross U, et al. Monocyte chemotactic protein-1 secretion and expression after *Toxoplasma gondii* infection in vitro depend on the stage of the parasite. FEMS Microbiol Lett 2002;214(1):45–9.
[19] Luppi P. How immune mechanisms are affected by pregnancy. Vaccine 2003; 21(24):3352–7.
[20] Schafer-Somi S. Cytokines during early pregnancy of mammals: a review. Anim Reprod Sci 2003;75(1–2):73–94.
[21] Hanna N, Hanna I, Hleb M, Wagner E, Dougherty J, Balkundi D, et al. Gestational age-dependent expression of IL-10 and its receptor in human placental tissues and isolated cytotrophoblasts. J Immunol 2000;164(11):5721–8.
[22] Vats V, Agrawal T, Salhan S, Mittal A. Primary and secondary immune responses of mucosal and peripheral lymphocytes during *Chlamydia trachomatis* infection. FEMS Immunol Med Microbiol 2007;49(2):280–7.
[23] Sharma A, Rajappa M, Saxena A, Sharma M. Cytokine profile in Indian women with cervical intraepithelial neoplasia and cancer cervix. Int J Gynecol Cancer 2007;17(4):879–85.
[24] Mor G. Inflammation and pregnancy: the role of toll-like receptors in trophoblast-immune interaction. Ann N Y Acad Sci 2008;1127:121–8.
[25] Jones RL, Hannan NJ, Kaitu'u TJ, Zhang J, Salamonsen LA. Identi-fication of chemokines important for leukocyte recruitment to the human endometrium at the times of embryo implantation and menstruation. J Clin Endocrinol Metab 2004;89:6155–67.
[26] Dunn D, Wallon M, Peyron F, Petersen E, Peckham C, Gilbert R. Mother-to-child transmission of toxoplasmosis: risk estimates for clinical counselling. Lancet 1999;353(9167):1829–33.
[27] Bonecchi R, Bianchi G, Bordignon PP, D'Ambrosio D, Lang R, Borsatti A, et al. Differential expression of chemokine receptors and chemotactic responsiveness of type 1 T helper cells (Th1s) and Th2s. J Exp Med 1998;187(1):129–34.
[28] Van Coillie E, Van Damme J, Opdenakker G. The MCP/eotaxin subfamily of CC chemokines. Cytokine Growth Factor Rev 1999;10(1):61–86.
[29] Del Rio L, Butcher BA, Bennouna S, Hieny S, Sher A, Denkers EY. *Toxoplasma gondii* triggers myeloid differentiation factor 88-dependent IL-12 and chemokine ligand 2 (monocyte chemoattractant protein 1) responses using distinct parasite molecules and host receptors. J Immunol 2004;172(11): 6954–60.
[30] Kallikourdis M, Andersen KG, Welch KA, Betz AG. Alloantigen-enhanced accumulation of CCR5+ 'effector' regulatory T cells in the gravid uterus. Proc Natl Acad Sci U S A 2007;104(2):594–9.
[31] Vassiliadou N, Tucker L, Anderson DJ. Progesterone-induced inhibition of chemokine receptor expression on peripheral blood mononuclear cells correlates with reduced HIV-1 infectability in vitro. J Immunol 1999;162(12): 7510–8.

[32] Aliberti J, Valenzuela JG, Carruthers VB, Hieny S, Andersen J, Charest H, et al. Molecular mimicry of a CCR5 binding-domain in the microbial activation of dendritic cells. Nat Immunol 2003;4(5):485–90.
[33] Gopal R, Birdsell D and Monroy FP. Regulation of chemokine responses in intestinal epithelial cells by stress and Toxoplasma gondii infection. Parasite Immunol;33(1):12–24.
[34] Aviles H, Stiles J, O'Donnell P, Orshal J, Leid J, Sonnenfeld G, et al. Kinetics of systemic cytokine and brain chemokine gene expression in murine toxoplasma infection. J Parasitol 2008;94(6):1282–8.
[35] Sacks GP, Redman CW, Sargent IL. Monocytes are primed to produce the Th1 type cytokine IL-12 in normal human pregnancy: an intracellular flow cytometric analysis of peripheral blood mononuclear cells. Clin Exp Immunol 2003;131(3):490–7.
[36] Chaves AC, Ceravolo IP, Gomes JA, Zani CL, Romanha AJ, Gazzinelli RT. IL-4 and IL-13 regulate the induction of indoleamine 2,3-dioxygenase activity and the control of *Toxoplasma gondii* replication in human fibroblasts activated with IFN-gamma. Eur J Immunol 2001;31(2):333–44.
[37] Gazzinelli RT, Eltoum I, Wynn TA, Sher A. Acute cerebral toxoplasmosis is induced by in vivo neutralization of TNF-alpha and correlates with the down-regulated expression of inducible nitric oxide synthase and other markers of macrophage activation. J Immunol 1993;151(7):3672–81.
[38] Deckert-Schluter M, Bluethmann H, Rang A, Hof H, Schluter D. Crucial role of TNF receptor type 1 (p55), but not of TNF receptor type 2 (p75), in murine toxoplasmosis. J Immunol 1998;160(7):3427–36.
[39] Yap GS, Scharton-Kersten T, Charest H, Sher A. Decreased resistance of TNF receptor p55- and p75-deficient mice to chronic toxoplasmosis despite normal activation of inducible nitric oxide synthase in vivo. J Immunol 1998;160(3):1340–5.
[40] Scharton-Kersten T, Denkers EY, Gazzinelli R, Sher A. Role of IL12 in induction of cell-mediated immunity to *Toxoplasma gondii*. Res Immunol 1995;146(7–8):539–45.
[41] Moore KW, de Waal Malefyt R, Coffman RL, O'Garra A. Interleukin-10 and the interleukin-10 receptor. Annu Rev Immunol 2001;19:683–765.
[42] Barbosa BF, Silva DA, Costa IN, Mineo JR, Ferro EA. BeWo trophoblast cell susceptibility to *Toxoplasma gondii* is increased by interferon-gamma, interleukin-10 and transforming growth factor-beta1. Clin Exp Immunol 2008;151(3):536–45.
[43] Wille U, Villegas EN, Striepen B, Roos DS, Hunter CA. Interleukin-10 does not contribute to the pathogenesis of a virulent strain of *Toxoplasma gondii*. Parasite Immunol 2001;23(6):291–6.
[44] Fuentes I, Rubio JM, Ramirez C, Alvar J. Genotypic characterization of *Toxoplasma gondii* strains associated with human toxoplasmosis in Spain: direct analysis from clinical samples. J Clin Microbiol 2001;39(4):1566–70.
[45] Ajzenberg D, Cogne N, Paris L, Bessieres MH, Thulliez P, Filisetti D, et al. Genotype of 86 *Toxoplasma gondii* isolates associated with human congenital toxoplasmosis, and correlation with clinical findings. J Infect Dis 2002;186(5):684–9.